Zinc absorption and kinetics during pregnancy and lactation in Brazilian women[1–4]

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ABSTRACT

Background: Adjustments in zinc absorption and endogenous excretion maintain zinc homeostasis in nonpregnant adults fed low-zinc diets. The effects on zinc homeostasis of a low zinc intake during pregnancy and lactation have not been described in a longitudinal study.

Objective: We examined longitudinal changes in fractional zinc absorption (FZA) and zinc kinetics in 10 healthy Brazilian women who habitually consumed a marginal zinc diet (≈9 mg Zn/d).

Design: Zinc status was measured at 10–12 (early pregnancy; EP) and 34–36 (late pregnancy; LP) wk of pregnancy and at 7–8 wk after delivery (early lactation; EL). Zinc kinetics and FZA were studied by using stable isotopic tracers.

Results: Zinc intake averaged 9 ± 3 mg/d throughout the study. FZA increased from 29 ± 6% at EP to 43 ± 10% at LP and to 39 ± 13% at EL ($P < 0.05$). FZA was inversely related to plasma zinc at EL ($r = -0.73$, $P = 0.02$) and LP ($r = -0.72$, $P = 0.07$). Plasma zinc mass was 23% greater at LP than at EP or EL ($P < 0.05$). The amount of zinc (mg/d) that fluxed between plasma and the most-rapidly-turning-over extravascular pool was 53% greater at LP than at EP or EL ($P < 0.05$). The zinc flux between plasma and the less-rapidly-turning-over zinc pool at EL was 27% greater than that at EP or LP, but this difference was not significant.

Conclusions: FZA increased significantly in women with marginal zinc intakes during pregnancy and lactation; the increase was higher in women with low plasma zinc. Plasma zinc was distributed into a different exchangeable pool at LP than at EL. *Am J Clin Nutr* 2005;82:118–24.

KEY WORDS Pregnancy, lactation, zinc absorption, zinc kinetics, stable isotopes, Brazilian women

INTRODUCTION

The need for zinc increases during pregnancy and lactation because of the greater demands of normal embryogenesis, fetal growth, and milk secretion. The total demand in a full-term pregnancy is ≈100 mg Zn; the need parallels fetal growth and reaches a peak increase of 1 mg absorbed Zn/d in the third trimester (1). During lactation, the additional demand for absorbed zinc is highest in the first 2 mo of lactation, when milk zinc concentrations are high (2). These increased zinc needs could be met by an increase in zinc intake or by adjustments in zinc homeostasis. Because women do not typically increase their zinc intake during pregnancy and lactation (3), adjustments in zinc absorption, excretion, tissue distribution, or all 3 must occur to meet these greater demands for zinc.

The efficiency of zinc absorption has been measured in pregnant (4) and lactating (5–7) women with marginal zinc intakes (<10 mg/d) and in women consuming ≈15 mg Zn/d during pregnancy and lactation (8). These studies showed that the efficiency of zinc absorption increased during lactation, especially among women with marginal zinc intakes. However, the effect of pregnancy on the efficiency of zinc absorption was inconclusive.

Additional homeostatic adjustments, such as reduced urinary zinc excretion, reduced endogenous fecal zinc losses, and an increased mobilization of zinc from bone and other tissues, could theoretically contribute to the greater need for zinc during pregnancy and lactation in women with low zinc intakes (3, 9, 10). However, studies of the effects of pregnancy and lactation on these mechanisms are limited. A study in China showed that intestinal conservation of endogenous zinc was a major factor in achieving zinc homeostasis in lactating women who consumed only 7.6 mg Zn/d (7). A preliminary zinc kinetic study of lactating women of the Amazon River Valley (Brazil) who consumed diets providing 8.4 mg Zn/d suggested that the lactation state increased the plasma zinc turnover rate and decreased the size of the exchangeable zinc pool in these women (6). The development of detailed compartmental models of zinc metabolism in nonpregnant, nonlactating women by using oral and intravenous stable isotopes provided a means of conducting more detailed studies of zinc homeostasis during pregnancy and lactation (11). Therefore, the purpose of the current study was to ascertain

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[2] Presented in part at Experimental Biology 2002 (New Orleans, LA, April 20–24, 2002) and at the 11th International Symposium on Trace Elements in Man and Animals (TEMA 11, Berkeley, CA, June 2–6, 2002).

[3] Supported by the Center for Research and Information in Nutrition, Paris, France, and by a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (to CMD).

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Received December 20, 2004.

Accepted for publication March 14, 2005.

changes in fractional zinc absorption (FZA), urinary zinc excretion, and zinc kinetics by using a 3-compartment model during pregnancy and lactation in a group of healthy Brazilian women who habitually consumed a diet with marginal zinc content.

SUBJECTS AND METHODS

Subjects

Subjects were recruited for the study at their first prenatal visit at the Maternidade Escola of the Federal University of Rio de Janeiro, Brazil. Ten adult women of low socioeconomic status agreed to participate in the study. The women were healthy, nonsmoking, physically inactive multigravidae, and they had had no health problems or complications during pregnancy. All of the women were of mixed black and white race. Iron supplements (50–100 mg Fe/d) were given to all of the women during the second half of pregnancy (22–35 wk of gestation) as part of their routine prenatal care, but none of the women took zinc or other vitamin or mineral supplements. No iron supplements were used after delivery. The women were advised to consume their usual diets throughout the study.

Written informed consent was obtained from all subjects. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and by the Ethical Committee of Maternidade Escola, Federal University of Rio de Janeiro.

Study design

Clinical studies of zinc homeostasis were done at 3 time points: week 10–12 (early pregnancy; EP) and week 34–36 (late pregnancy; LP) of pregnancy and 7–8 wk after delivery (early lactation; EL). Iron supplements were not taken during the clinical studies. All women were exclusively breastfeeding their infants at EL. Dietary zinc intake, fasting plasma and erythrocyte zinc concentrations, 24-h urinary zinc excretion, FZA, and zinc kinetics were measured at each time point.

Procedures and analysis

Dietary intake was assessed from weighed food-intake records kept by the subject on 3 consecutive days before the clinical study at each of the 3 time points. Subjects were carefully instructed and trained by one of the investigators (CVZ) in the food-weighing and -recording procedures. The dietary nutrient intake was estimated from the food records by using the Food Processor nutrient database (ESHA Research, Salem, OR) that was adapted for Brazilian foods with the use of published food-composition data (12). Phytate intake was estimated from published food phytate data (13).

Studies of FZA, plasma zinc kinetics, and urinary zinc excretion were performed after an overnight (10–12 h) fast at the Maternidade Escola of the Federal University of Rio de Janeiro by using a double isotopic tracer technique (8, 11). Highly enriched stable isotopic tracers, ie, $^{67}Zn_{tr}$ (90.1% enriched with ^{67}Zn) and $^{70}Zn_{tr}$ (88.5% enriched with ^{70}Zn) as zinc oxide (Oak Ridge National Laboratory, Oak Ridge, TN), were dissolved in concentrated hydrogen chloride (Optima; Fisher, Pittsburgh, PA; 3 μL HCl/mg ZnO). They were then diluted with triply deionized water to a final concentration of 1.0 mg $^{67}Zn_{tr}$/mL or 0.4 mg $^{70}Zn_{tr}$/mL and filtered through a 0.22-μm filter unit (Millipore Products Division, Bedford, MA). The solution for intravenous use was sterilized and pyrogen tested by the School of Pharmacy, University of California, San Francisco. Intravenous doses (1.0 mL) containing 0.4 mg $^{70}Zn_{tr}$ were stored in individually sealed, sterile vials until they were used. The amounts of administered oral and intravenous tracer doses were calculated from the total zinc concentration of the solutions, which was measured by using atomic absorption flame spectrometry and the weight of the administered dose.

On the morning of the clinical study, the weight and height of the subjects were recorded, and an indwelling catheter was placed in the antecubital vein, from which a baseline (fasting) blood sample and all other blood samples were drawn with Monovette syringes (Sarstedt, Hayward, CA) that contained heparin-coated beads. The subjects then received a standard breakfast consisting of French bread (50 g), butter (10 g), and whole milk (50 mL) mixed with coffee (50 mL); this was followed by the ingestion of 1 mg $^{67}Zn_{tr}$ in 50 mL water. The cup with the stable isotopic tracer solution was rinsed 3 times with water, and the water was consumed. The total zinc intake (breakfast plus oral zinc tracer) was 1.22 mg, and the total molar ratio of phytate to zinc was <1. Immediately after breakfast, a "butterfly" infusion set was used to infuse 0.4 mg $^{70}Zn_{tr}$ (1.0 mL) over 1–2 min into the antecubital vein of the arm opposite that used for blood sampling. The butterfly tubing was flushed with 5 mL sterile saline solution to ensure that the entire tracer dose was infused. The exact amount of tracer solution infused was ascertained by weighing the syringe before and after the infusion. Blood samples (8 mL) were taken via the catheter at 4, 8, 12, 16, 20, 30, 45, and 60 min and 2, 3, 6, 9, 12, and 24 h after the $^{70}Zn_{tr}$ infusion. A complete 24-h urine collection was obtained on the test day. Samples from the first urinary void were collected in the morning on days 3, 4, and 5 after the test.

Blood samples were refrigerated at 4 °C immediately after being drawn, and the plasma was separated within 2 h. Erythrocytes were obtained from the fasting baseline samples by removal of the buffy coat layer of packed red blood cells, washing of the cells twice with ice-cold 0.9% saline, and centrifugation at 800 $\times$ *g* for 10 min at 4 °C. The supernatant fluid was discarded, and an equal volume of ice-cold deionized water was added to the erythrocytes and mixed. Urine samples were weighed, and aliquots were acidified to a pH of 2.0 with trace-metal grade hydrogen chloride (Fisher). Aliquots of plasma, erythrocyte lysates, and urine were stored at −20 °C until they were analyzed for zinc. We used the cyanomethemoglobin method to measure hemoglobin; atomic absorption spectrometry to measure plasma, erythrocyte, and urinary zinc (8); a modified Lowry method to measure protein in erythrocytes (14); and the cadmium-hemoglobin affinity assay to measure metallothionein in erythrocytes (15).

Procedures used to prepare and analyze the urinary samples for mass spectrometric analysis by using inductively coupled plasma (ICP) were the same as those used previously in our laboratory (8). Briefly, urinary zinc was purified by ion exchange chromatography and submitted to ICP mass spectrometry (MS) (Sciex ELAN 5000 ICP-MS; Perkin Elmer, Norwalk, CT) for determination of the isotopic ratios of ^{67}Zn to ^{66}Zn and of ^{70}Zn to ^{66}Zn. The isotopic ratios were then converted to tracer-tracee ratios (TTRs) as previously described (11), in which the TTR data highly enriched in ^{67}Zn and ^{70}Zn can be defined as $^{67}Zn_{TTR}$ and $^{70}Zn_{TTR}$, respectively.

FZA was measured by a novel modification of the double isotopic tracer ratio technique in urine as previously described (16, 17). Briefly, this technique is used to estimate FZA from spot urine samples obtained 3, 4, and 5 d after simultaneous oral and intravenous tracer administration. The 3 FZA determinations are then averaged for the reported result. Our current technique for FZA measurement, a slight modification of the above, takes advantage of the observation that both oral and intravenous TTR measurements ($^{67}Zn_{TTR}$ and $^{70}Zn_{TTR}$, respectively) disappear from the plasma by the same fractional loss rate, ie, those measurements are describable by parallel lines on a semi-log plot when simple regression lines are fitted to the TTR data. The same parallel regression line pattern is also observed in sequential sampling of $^{67}Zn_{TTR}$ and $^{70}Zn_{TTR}$ data from spot urine samples. This technique is implemented by using the SAAM II computer program (version 1.2; SAAM Institute, Seattle, WA) in which individual exponential equations are fitted simultaneously to the spot urine $^{67}Zn_{TTR}$ and $^{70}Zn_{TTR}$ data, subject to the constraint that the exponential components are equal. Thus, the individual exponential equation for $^{67}Zn_{TTR}(t)$ is

$$^{67}Zn_{TTR}(t) = {}^{67}Int_{TTR}e^{-k^{67}t} \quad (1)$$

and that for $^{70}Zn_{TTR}(t)$ is

$$^{70}Zn_{TTR}(t) = {}^{70}Int_{TTR}e^{-k^{70}t} \quad (2)$$

where $^{67}Int_{TTR}$ and $^{70}Int_{TTR}$ are the intercepts of the exponential equations describing the regression lines through the $^{67}Zn_{TTR}$ and $^{70}Zn_{TTR}$ data from spot urine over days 3–5, and where $k^{70} = k^{67}$.

FZA is then given by the equation

$$FZA = (^{67}Int_{TTR}/dose^{67}Zn_{tr})/(^{70}Int_{TTR}/dose^{70}Zn_{tr})$$
$$= (^{67}Int_{TTR}/^{70}Int_{TTR}) \times (dose^{70}Zn_{tr}/Dose^{67}Zn_{tr}) \quad (3)$$

Plasma samples were prepared for stable isotope analysis as done previously (11). TTRs in the plasma were calculated from isotopic ratios of ^{67}Zn to ^{66}Zn and of ^{70}Zn to ^{66}Zn that were measured by using ICP-MS. Plasma zinc concentrations, measured by ICP at each sampling time over 24 h, were corrected for the tracer mass $^{67}Zn_{tr}$ and $^{67}Zn_{tr}$ as described previously (11) and averaged for the best estimate of plasma zinc concentration. A 3-compartment model was fitted to the plasma $^{70}Zn_{tr}$ data over 24 h by using the SAAM II modeling software (**Figure 1**). The 3 kinetically distinct zinc compartments of the model were Q_1, which represented the plasma zinc pool; Q_2, which represented a rapidly-turning-over tissue zinc pool; and Q_3, which represented a more-slowly-turning-over tissue zinc pool.

The values for the rate constants (ie, $k_{i,j}$) and their uncertainties were ascertained from the model at the least-squares fit. The steady-state solution of the model, calculated by assuming a known value (mg) for Q_1 and by assuming that all entry and irreversible loss of zinc occurred through the plasma compartment, provided estimates (in mg) of Q_2 and Q_3 as well as estimates (in mg/h) of all mass fluxes (R_{ij}) between compartments.

The Q_1 mass was calculated by multiplying the estimated total plasma volume by the measured plasma zinc concentration. Plasma volume (mL) was calculated from body surface area (m^2) multiplied by 1440, with the 50% predicted increase in plasma volume during pregnancy being taken into account (18). We assumed that the plasma volume increased by 50% from EP to LP. The exchangeable zinc pool, calculated as the sum of Q_1, Q_2, and Q_3, represented the total space in which the isotopic tracer equilibrated in 24 h.

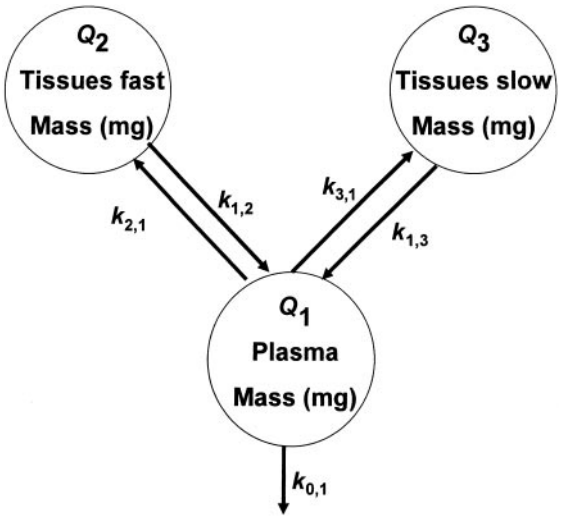

FIGURE 1. Three-compartment model of 24-h zinc kinetics, in which the circles represent kinetically distinct zinc compartments. Q_1 represents the plasma pool, and Q_2 and Q_3 represent a rapidly-turning-over and a slowly-turning-over tissue zinc pool, respectively, that exchange bidirectionally with the plasma pool. Transfer rate constants of the zinc movement between compartments are represented as $k_{i,j}$, which denotes the fractional rate of transfer of zinc from pool j to pool i per hour. $k_{0,1}$ represents the constant rate of transfer from Q_1 out of the system.

Statistical analysis

Statistical analysis was performed by using STATGRAPHICS software (version 7; Manugistics, Cambridge, MA). Longitudinal comparisons were done by using repeated-measures analysis of variance. Pairwise significant differences were assessed by using Tukey's range test. Associations between variables were examined by simple correlation analysis. Values were considered significant at $P < 0.05$.

RESULTS

The women were healthy, had appropriate weight-for-height, and had a normal blood hemoglobin concentration in EP (**Table 1**). All women had normal length of gestation (37–42 wk) except one woman, who had a premature delivery at 31 wk gestation. Gestational weight gains were between 6.5 and 18.9 kg. The

TABLE 1
Characteristics of the women studied[1]

	Value
Age (y)	30 ± 4
Prepregnancy BMI (kg/m^2)	23 ± 3
Parity	2.6 ± 1.6
Early-pregnancy blood hemoglobin (g/L)	121 ± 10
Length of gestation (wk)	39 ± 2
Weight gain during gestation (kg)	11.4 ± 5.8
Infant birth weight (kg)	3.1 ± 0.6
Infant birth length (cm)	51 ± 2

[1] All values are $\bar{x}$ ± SD; $n = 9$ (the 10th woman in the study delivered prematurely; *see* text).

TABLE 2
Dietary intakes from 3-d weighed-food records during pregnancy and lactation[1]

	EP	LP	EL
Energy (kcal/d)	1910 ± 660	2130 ± 400	2190 ± 610
Protein (g/d)	69 ± 30	79 ± 26	70 ± 21
Zinc (mg/d)	9 ± 5	9 ± 2	9 ± 3
Iron (mg/d)	13 ± 6	14 ± 5	14 ± 4
Phytate (mg/d)	1290 ± 660	1680 ± 840	1770 ± 930
Molar phytate:zinc	16 ± 8	19 ± 7	18 ± 9
Dietary fiber (g/d)	20 ± 9	23 ± 8	20 ± 6

[1] All values are $\bar{x}$ ± SD; $n = 10$. EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk).

women gave birth to healthy infants who weighed 2.1 kg (premature delivery) and from 2.7 to 4.4 kg (term deliveries) at birth and who gained weight normally during the lactation period.

The women habitually consumed diets that had marginal zinc content (**Table 2**). Over the entire study, mean dietary zinc intake was 8.9 mg/d, and mean molar phytate:zinc was 17.4. Beans, rice, corn meal, and manioc flour were the major sources of dietary phytate. Dietary intakes of energy, protein, zinc, iron, fiber, and phytate did not change significantly during the study.

Plasma zinc concentrations decreased 25% from EP to LP ($P < 0.01$) and returned to the EP concentration at EL (**Table 3**). Erythrocyte zinc was significantly higher at LP and EL than at EP ($P < 0.02$). Erythrocyte metallothionein did not change significantly during the study.

Urinary zinc did not change significantly during pregnancy and lactation (Table 3). FZA was 49% and 37% higher at LP and EL, respectively, than at EP ($P < 0.03$). FZA was inversely related to plasma zinc concentrations at LP, but the relation was not significant ($r = -0.718$, $P = 0.069$). A significant inverse relation was evident at EL ($r = -0.732$, $P = 0.016$) (**Figure 2**).

The kinetic values measured varied significantly between subjects (**Table 4**). There were no significant changes during the study in any of the rate constants, but some trends were apparent. Compared with EP, the rate of zinc movement at LP from Q_1 to Q_2 ($k_{2,1}$) tended to be higher, whereas that from Q_1 to Q_3 ($k_{3,1}$) tended to be lower. In contrast, at EL, the rate of zinc movement

TABLE 3
Biochemical zinc indexes, urinary zinc excretion, and fractional zinc absorption (FZA) during pregnancy and lactation[1]

	EP ($n = 10$)	LP ($n = 9$)	EL ($n = 10$)
Plasma zinc (μmol/L)	11.4 ± 1.7[a]	8.5 ± 0.7[b]	11.2 ± 3.5[a]
Erythrocyte zinc (μmol/g protein)	0.50 ± 0.07[a]	0.54 ± 0.05[b]	0.57 ± 0.11[b]
Erythrocyte metallothionein (nmol/g protein)	2.9 ± 0.6	2.9 ± 0.5	3.2 ± 0.6
Urinary zinc (μmol/d)	8.4 ± 3.1	9.5 ± 3.5	8.6 ± 4.3
FZA[2]	0.29 ± 0.06[a]	0.43 ± 0.10[b]	0.39 ± 0.13[b]

[1] All values are $\bar{x}$ ± SD. EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk). Means in a row with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA and Tukey's test).

[2] Because of analytic problems with 1 subject at LP, longitudinal comparison of FZA involved only 8 subjects.

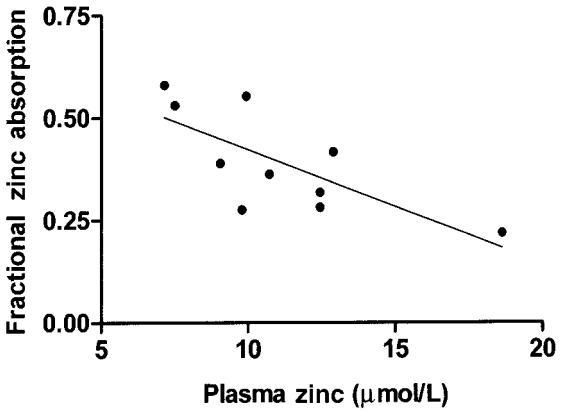

FIGURE 2. Relation by simple correlation analysis between fractional zinc absorption and plasma zinc at early lactation ($n = 10$) ($r = -0.73$, $P = 0.02$). The regression equation is fractional zinc absorption (%) = 0.702 − 0.028 plasma zinc (μmol/L).

from Q_1 to Q_2 tended to be lower and that from Q_1 to Q_3 tended to be higher than the same movements at EP.

The Q_1 mass increased ≈23% from EP to LP ($P < 0.05$) and returned to the EP mass at EL (Table 4). There were no significant changes in the mass in the other compartments. However, compared with EP, the trend at LP was toward a greater mass in Q_2 and a smaller mass in Q_3, and the trend at EL was toward a smaller mass in Q_2 and a greater mass in Q_3.

The exchangeable zinc pool (the sum of Q_1, Q_2, and Q_3) did not change significantly during pregnancy and lactation. Zinc flux from plasma (Q_1) to the most-rapidly-turning-over extravascular pool (Q_2) (ie, $R_{2,1}$) increased ≈53% from EP to LP ($P < 0.05$) (Table 4). Zinc flux from plasma (Q_1) into the less-rapidly-turning-over pool (Q_3) (ie, $R_{3,1}$) increased ≈27% from EP to EL, although this increase was not significant. These changes represent the movement of an additional 75 mg Zn/d from

TABLE 4
Zinc kinetic parameters of the 3-compartment model during pregnancy and lactation[1]

	EP ($n = 10$)	LP ($n = 9$)	EL ($n = 10$)
Rate constant (h^{-1})			
$k_{0,1}$	1.0 ± 0.5	0.9 ± 0.5	0.9 ± 0.3
$k_{2,1}$	3.4 ± 1.2	4.4 ± 2.3	3.1 ± 1.3
$k_{1,2}$	1.4 ± 0.8	2.0 ± 1.5	3.7 ± 3.4
$k_{3,1}$	3.6 ± 1.2	3.1 ± 1.7	4.4 ± 1.5
$k_{1,3}$	0.12 ± 0.06	0.2 ± 0.3	0.16 ± 0.04
Pool size (mg)			
Q_1	1.7 ± 0.3[a]	2.1 ± 0.3[b]	1.8 ± 0.3[a]
Q_2	4.9 ± 2.7	5.8 ± 2.6	3.8 ± 4.1
Q_3	45 ± 13	41 ± 19	52 ± 22
EZP (mg)	52 ± 13	49 ± 19	58 ± 23
Flux (mg/h)			
$R_{0,1}$	1.6 ± 0.8	1.7 ± 0.7	1.5 ± 0.6
$R_{2,1}$	5.7 ± 1.7[a]	8.7 ± 3.3[b]	5.6 ± 3.1[a]
$R_{3,1}$	6.2 ± 2.8	6.0 ± 2.4	7.9 ± 30

[1] All values are $\bar{x}$ ± SD. EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk); Q_1, plasma zinc pool; Q_2, a rapidly-tuning-over tissue zinc pool; Q_3, a more-slowly-tuning-over tissue zinc pool; EZP, exchangeable zinc pool (the sum of Q_1, Q_2, and Q_3). Means in a row with different superscript letters are significantly different, $P < 0.05$ (repeated-measures analysis of variance and Tukey's test).

TABLE 5
Total zinc absorbed from the test meal in the 2 longitudinal studies of fractional zinc absorption (FZA) during pregnancy and lactation

	Zinc intake at measurement of absorption			Period of the study					
				Before pregnancy or early pregnancy		Late pregnancy		Early lactation	
Study	Test meal	Oral isotope	Total	FZA	Zinc absorbed	FZA	Zinc absorbed	FZA	Zinc absorbed
	mg	*mg*	*mg*	*%*	*mg*	*%*	*mg*	*%*	*mg*
Fung et al (8)	1.22[1]	3.0	4.22	14.6[2]	0.62	19.4	0.82	25.3	1.07
Current study	0.22	1.0	1.22	28.7[3]	0.35	42.8	0.52	39.3	0.48

[1] EB Fung, personal communication, September 2003.
[2] Before pregnancy.
[3] Early pregnancy.

plasma into Q_2 at LP and of an additional 40 mg Zn/d from plasma into Q_3 at EL.

DISCUSSION

The adaptation of zinc homeostasis to pregnancy and lactation in women who habitually consume marginal zinc diets has been addressed in cross-sectional studies (4–7). To our knowledge, this is the first longitudinal study of intestinal zinc absorption and zinc kinetics during pregnancy and lactation in women with marginal dietary zinc intake (≈9 mg/d) and a moderate molar phytate:zinc (<20). The women did not change their habitual dietary intake during the study, which made it possible to study adjustments of zinc metabolism during pregnancy and lactation, when diet was essentially constant.

Although zinc intake was marginal, changes in plasma and erythrocyte zinc during pregnancy and lactation in the study subjects were similar to those described elsewhere in women with higher zinc intakes (1, 8, 19), which indicated normal physiologic zinc adjustments to these variables. Erythocyte metallothionein concentrations in these subjects were similar to those measured previously at delivery in women with higher zinc intakes (15). Pregnant women have significantly higher concentrations of erythrocyte metallothionein than do nonpregnant women (15), probably in response to the high concentrations of estrogen and progesterone during pregnancy (20).

Cross-sectional studies indicate that the efficiency of zinc absorption may be high during pregnancy and lactation in women with diets that are low in zinc (4, 6, 7). In the current study, FZA measured from a standardized breakfast meal was 0.29 at EP; it increased ≈49% at LP and remained high at EL. These longitudinal results, taken together with results from previous cross-sectional studies done at lactation (6, 7), indicated that an increased efficiency of zinc absorption at LP and during lactation contributes to meeting the increased zinc needs during reproduction, when zinc intakes are marginal.

The pattern of change in FZA during pregnancy and lactation in the women in the current study who were consuming ≈9 mg Zn/d was similar to that in the women who were consuming ≈15 mg Zn/d (8); there were higher rates of absorption at LP and EL, when the need for zinc for reproduction is high. The rates of absorption were significantly higher among the women with lower usual zinc intakes than among the women with higher usual zinc intakes, eg, 43% and 19% at LP and 39% and 25% at EL, respectively (**Table 5**). These differences suggest that the efficiency of zinc absorption may be greater in pregnant and lactating women with low zinc intakes. However, differences between the amount of zinc fed in the test meals in the current study and in that of Fung et al (8) may account for the different rates of zinc absorption. Total zinc intake inversely affects the efficiency of the absorption of zinc from a test meal (21). The total zinc intake in the test meal in the current study was only 30% of that in the study of Fung et al (8). The lower amount of zinc in the test meal in the current study probably contributed to the higher efficiencies of absorption in the Brazilian women. The increase in efficiency was insufficient, however, to equalize the amount of zinc absorbed from the test meal in our study and that of Fung et al: total absorbed zinc was about 35% lower at LP and 55% lower at EL in the women in the current study than in those in the study by Fung et al. Further studies are needed to establish the independent effects of dietary zinc intake and maternal zinc status on the homeostatic adjustments in zinc absorption during pregnancy and lactation.

Iron supplementation during pregnancy and lactation may have an effect on zinc absorption. In cross-sectional studies done during pregnancy (4) and lactation (22), zinc absorption was significantly lower in iron-supplemented women than in nonsupplemented women. In a longitudinal study from before pregnancy to lactation (8), FZA did not increase significantly in women who took iron supplements during lactation. In all of these studies, the iron supplement was consumed during periods of measurement of zinc absorption, which favored gastrointestinal interaction between the iron supplement and the zinc tracer. In the current study, however, the women stopped taking the iron supplement during the clinical trial to reduce the potential interaction of iron with dietary and tracer zinc in the gastrointestinal tract. We previously showed that supplementation with iron (100 mg/d) as ferrous sulfate taken separately from meals for 8 wk did not affect the absorption of zinc from a test meal in nonpregnant women (23). However, the effect on zinc absorption and homeostasis of iron supplementation and iron status during pregnancy and lactation should be investigated further.

The zinc status of the woman appears to be an important determinant of the physiologic adjustments made to meet zinc demands during pregnancy and lactation (3). In the women in the current study, FZA was inversely but not significantly related to plasma zinc concentrations at LP and at EL. It is possible that lower maternal plasma zinc concentrations somehow signaled the intestinal mucosa to increase the efficiency of zinc absorption when zinc needs were elevated. The lower plasma zinc concentrations at LP (8.5 and 9.8 μmol/L) and EL (11.2 and 12.5

μmol/L) in the women in the current study than in those studied by Fung et al, respectively, are consistent with this hypothesis. Experimental human zinc-depletion studies have shown that FZA increases when zinc intakes, and possibly zinc status, are reduced (24). An inverse relation between intestinal zinc absorption and maternal plasma zinc concentrations may contribute to improved fetal growth and breast milk synthesis and secretion when maternal diets are marginal in zinc. This may explain, in part, why little or no association between maternal plasma zinc and infant birth weight has been observed in women of low socioeconomic status (25). Urinary zinc excretion did not increase during pregnancy in the women in the current study, whereas it did increase in other studies (1, 8). It is possible that renal zinc conservation contributed to zinc homeostasis in women consuming low-zinc diets. The addition of supplemental zinc to iron and folate supplements increased urinary zinc concentrations at LP in Peruvian women who were consuming ≈7 mg Zn/d (26). The role of renal function in zinc homeostasis during pregnancy and lactation should be investigated further.

Compartmental analysis of zinc tracer kinetics has been used to describe zinc metabolism in men (27, 28) and in nonpregnant, nonlactating women (11). We also used a short-term zinc kinetics model to describe the effect of low zinc intakes in pregnant rats (29). In that study, we found that the turnover rate of the exchangeable plasma zinc pool increased with marginal intakes. On the basis of this experience with zinc compartmental modeling in pregnant rats, we felt that zinc kinetic studies could provide valuable insights into zinc homeostasis among women who had marginal zinc intakes.

In the current study, a 3-compartment model was used to describe the sizes and turnover rates of 2 extravascular pools (Q_2 and Q_3) that exchanged with the plasma zinc pool (Q_1) over a 24-h period. The turnover time of compartment Q_2 was <1 h, and that of compartment Q_3 was <10 h. Previous studies in men (30, 31), nonpregnant women (32), and pregnant rats (29) suggested that Q_2 and Q_3 are located within the liver, erythrocytes, and kidney, and, when the subject is pregnant, they may include the fetus. Similar anatomical studies have not been done in lactating animals.

Although the rate constants between zinc pools did not change significantly during pregnancy and lactation in this study, there tended to be an increased rate of plasma transfer into Q_2 at LP and into Q_3 during lactation. The high demands for zinc during pregnancy and lactation appear to increase the rate at which zinc moves from plasma into rapidly-turning-over zinc pools, but the flux goes to a different pool during pregnancy than during lactation. At LP, there was a 53% increase in zinc flux from plasma into Q_2, as a result of the combined increase in the rate constant $k_{2,1}$ and of the Q_1 mass. On the basis of this increased flux in LP, we estimate that an additional 75 mg Zn/d was transferred into the rapidly-turning-over zinc pool, which possibly reflected the increased zinc demands of the fetus and of zinc metabolism in maternal tissues, such as liver and bone marrow, during LP. The zinc flux from the plasma into compartment Q_3 during EL was 27% greater than that during EP; this greater flux contributed an additional transfer of 40 mg Zn/d from plasma into this pool, which may include the mammary gland and may, therefore, increase the availability of zinc for milk synthesis.

In summary, the plasma zinc flux between 2 exchangeable pools in LP differed significantly from that in EL. But the size of the total exchangeable zinc pool and the irreversible flux of zinc out of the system ($R_{0,1}$) into very-slowly-turning-over zinc pools or as zinc excretory losses were unchanged during pregnancy and lactation. To meet the increased demand for zinc during LP, the increased transfer of 75 mg Zn/d from the plasma into a rapidly-turning-over zinc pool was augmented by a 49% increase in the efficiency of intestinal zinc absorption. During lactation, the efficiency of zinc absorption increased by 37%, and zinc transfer from the plasma to a less-rapidly-turning-over exchangeable zinc pool increased by 40 mg/d (which was not significant). Women with lower plasma zinc concentrations had higher efficiencies of zinc absorption in EL. These results show that the homeostatic adjustments in zinc metabolism to meeting the demands of fetal growth differ from those needed to meet the demands of milk synthesis.

We thank the medical and nursing staffs of Maternidade Escola of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, for their valuable support during the clinical part of the study. We are very grateful to all the women who participated in this study.

CMD was responsible for the supervision of the study and writing of the manuscript and was involved in study design and data analysis. CLVZ was responsible for the clinical study and for data collection and analysis. LWR participated in management and laboratory analysis. DMS and RM conducted the estimates of fractional zinc absorption and zinc kinetics. JCK was responsible for the conception and funding of the study and was involved in study design and writing of the manuscript. All authors reviewed the manuscript. None of the authors had a personal or financial conflict of interest.

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